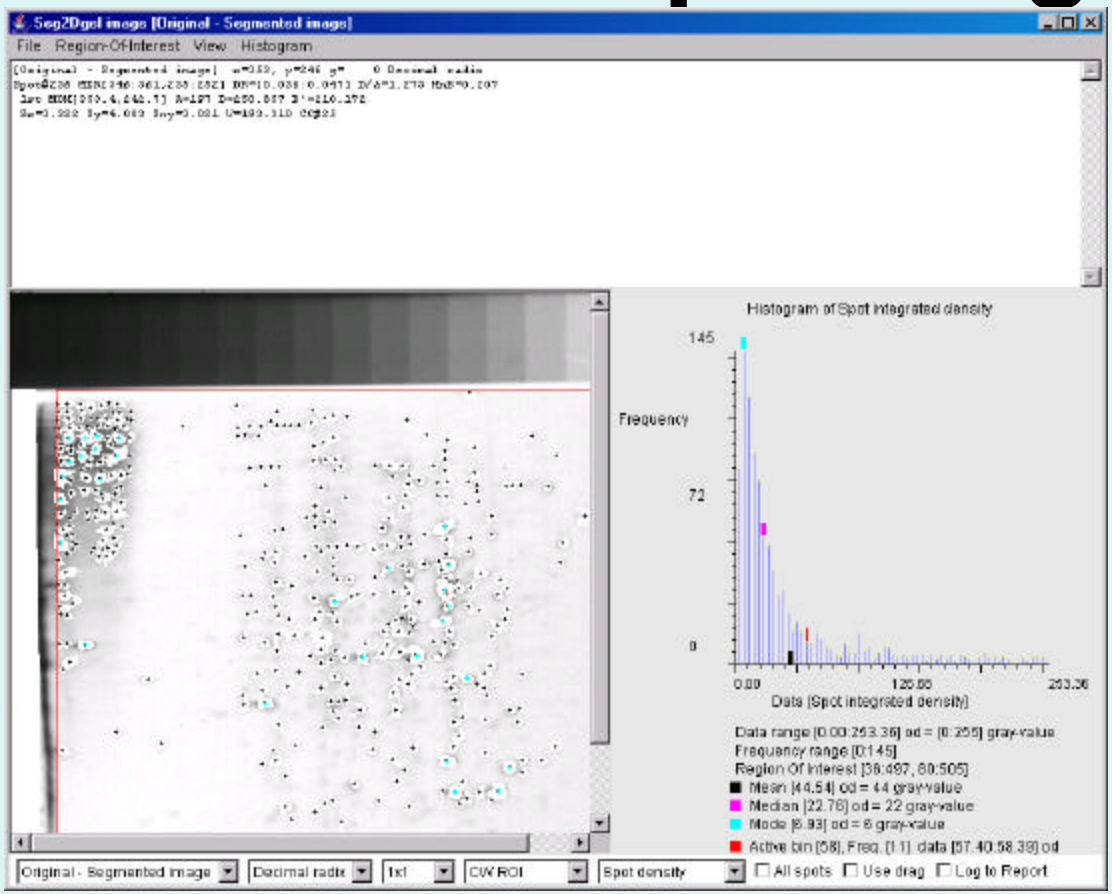
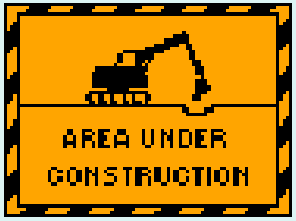
Seg2Dgel - 2D Electrophoretic Gel Spot Segmenter





http://open2dprot.sourceforge.net/Seg2Dgel

Revised: 12-03-2004, P. Lemkin, NCI-Frederick

Overview

- Seg2Dgel is an open-source Java 2D electrophoresis gel segmentation program for finding and measuring the integrated density and position of spots in a gel. It is a step [2] module in the pipeline analysis for the Open2Dprot project.

- The segmentation is performed on a computing window region of interest (ROI) of a 2D gel image file. It uses the second derivative (laplacian) magnitude and direction of the gaussian-smoothed gel image as well as neighborhood connectivity properties in determining spot extents.

- It handles grayscale TIFF, JPEG and GIF images.

- Images with calibrated grayscale (optical density, counts/min, etc.) may be used if calibrations are available.

Overview - GUI Window (2)

- Spot lists are generated in XML, tab-delimited, or human readable formats.

- The program may be run either interactively (-gui) with a graphical user interface (GUI) or under an OS shell command to implement batch (-nogui).

- Options are specified as Unix-style command line ‘-’ prefixed input switches so the program is also usable under batch. These may be set by an Options Wizard window.

- In the GUI version, after the segmentation is finished, the user has the option of interactively viewing any of the images generated by the segmenter with the Image Viewer window.

Overview - GUI Window (3)

- It has been extended to also handle low resolution 2D LC-MS images consisting of long narrow spots
- The narrow spots are detected using a horizontal or vertical laplacian filter
- Additional spot sizing can be done using the expected aspect ratio Sx/Sy of spots.

Overview - Image Viewer Window (4)

- User may interactively view any of the images generated by the segmenter.
- Query images for quantified spot data or look at small numeric windows (3x3 to 21x21 pixels) of the image data in decimal or optical density (if calibrated).
- View horizontal and/or vertical image density slides at the selected pixel.
- Dynamic histograms of image density or selected spot features with rudimentary data filtering that is useful for setting spot threshold sizing parameters.

Overview - Downloading and Installing (4)

- The user may modify the input switch options and save the new options in a "Seg2Dgel.properties" file in the current project directory so that it may be used as the default switch options in subsequent running of the segmenter.

- You may currently download the pre-alpha version and install it on your computer.

- Currently, Seg2Dgel is hardwired to start with the demo gel and with the -gui switch. However, you can override this in the “Edit options” popup wizard.

- The home page contains links to some screen shots of the interactive Image Viewer GUI.

http://open2dprot.sourceforge.net/Seg2Dgel

Seg2Dgel - 2D electrophoresis gel segmenter pre-alpha version - Netscape

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open2dprot.sourceforge.net

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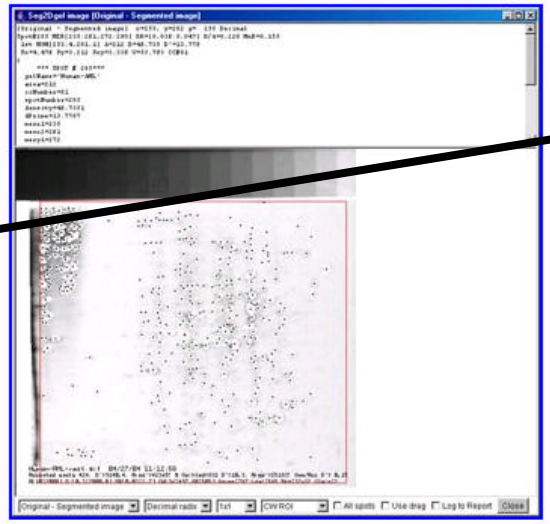
Seg2Dgel - 2D Gel Spot Segmenter

AREA UNDER CONSTRUCTION



Seg2Dgel - 2D electrophoresis gel spot segmenter pre-alpha version

Welcome To Seg2Dgel

http://open2dprot.sourceforge.net/Seg2Dgel and
http://www.lecb.ncifcrf.gov/Open2Dprot/Seg2Dgel/

Note: This pre-alpha level software is under construction and will change.
Not all functionality described is fully implemented or fully debugged.
This documentation will undergo revision as the program develops.

Introduction

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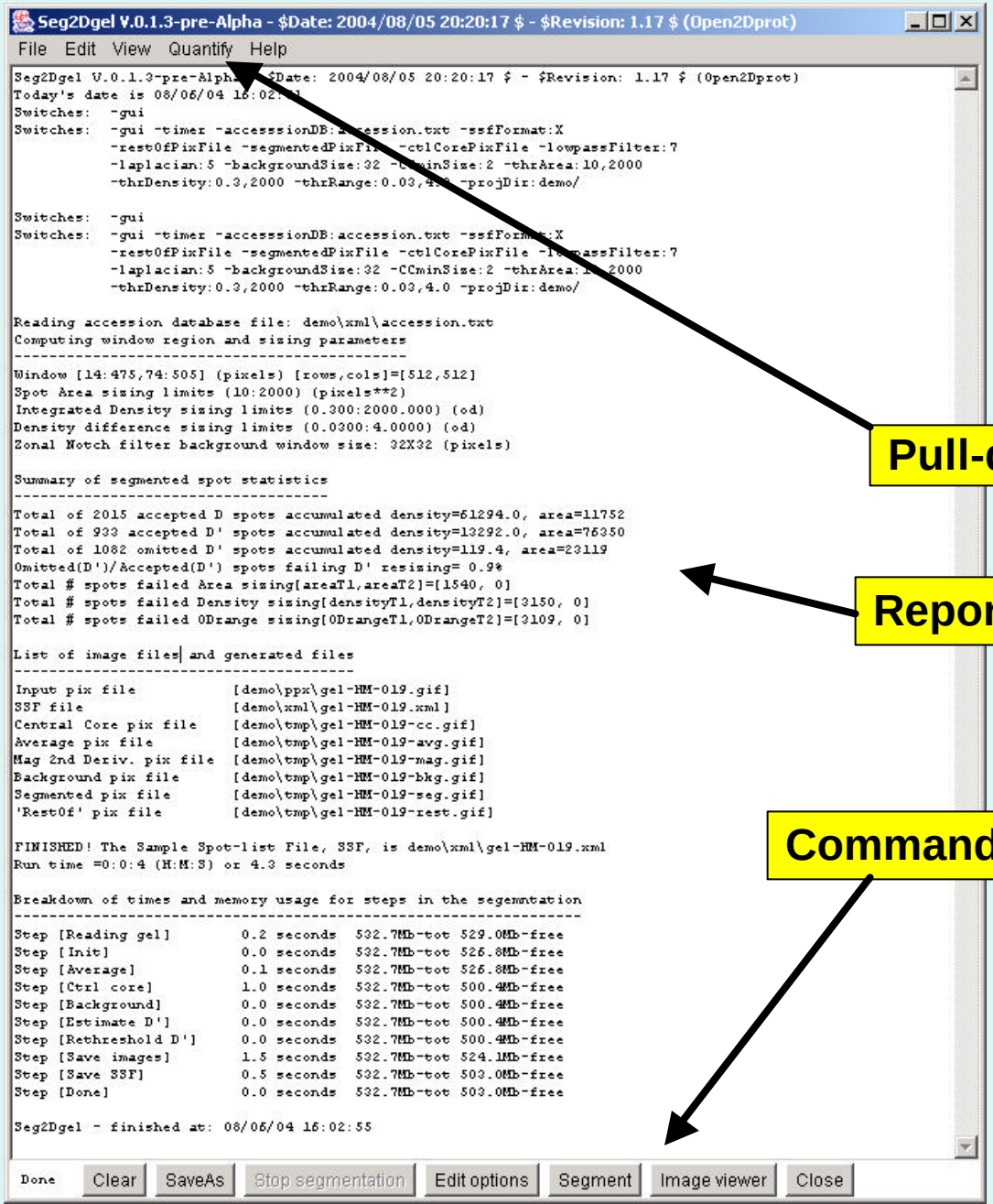
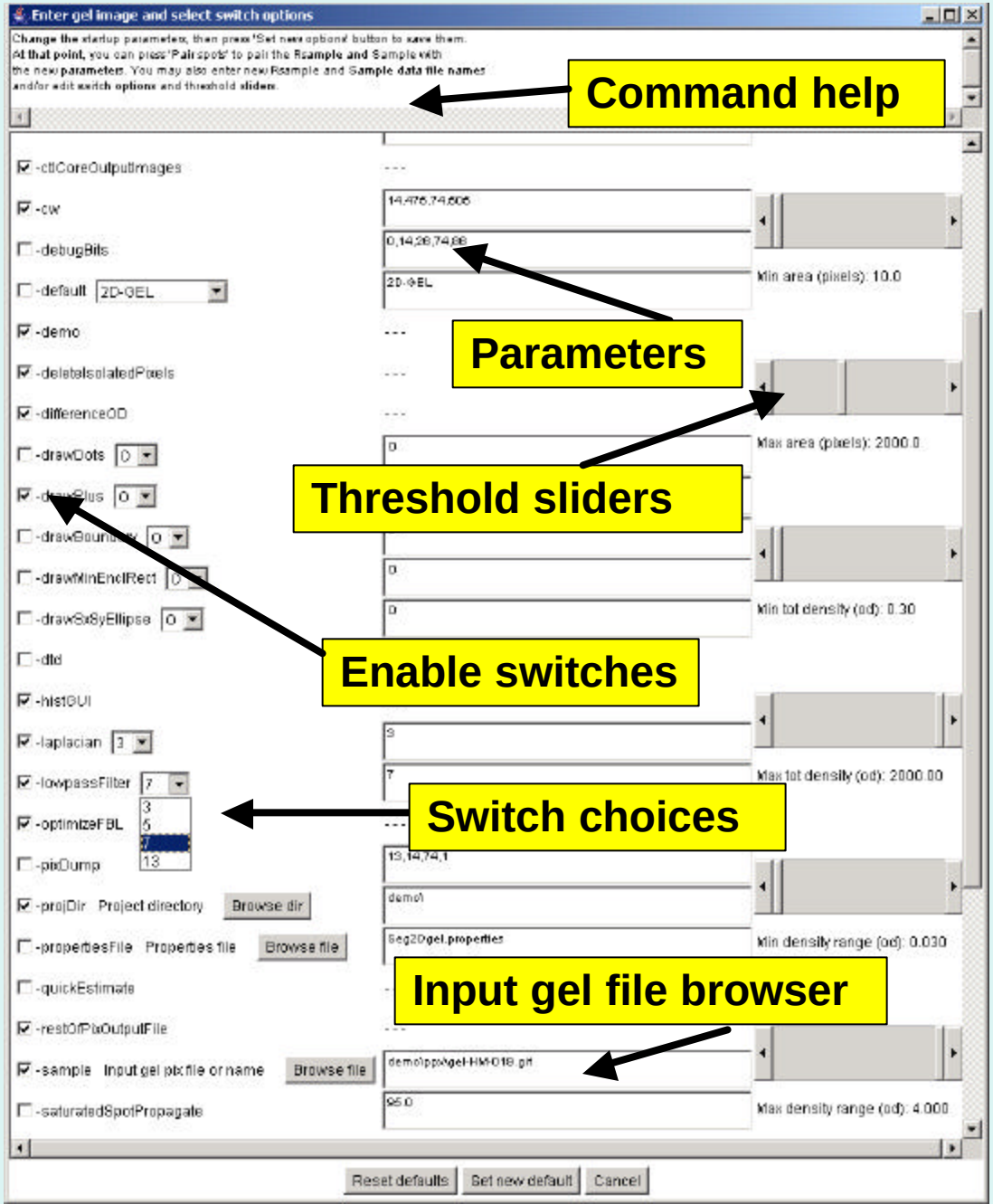
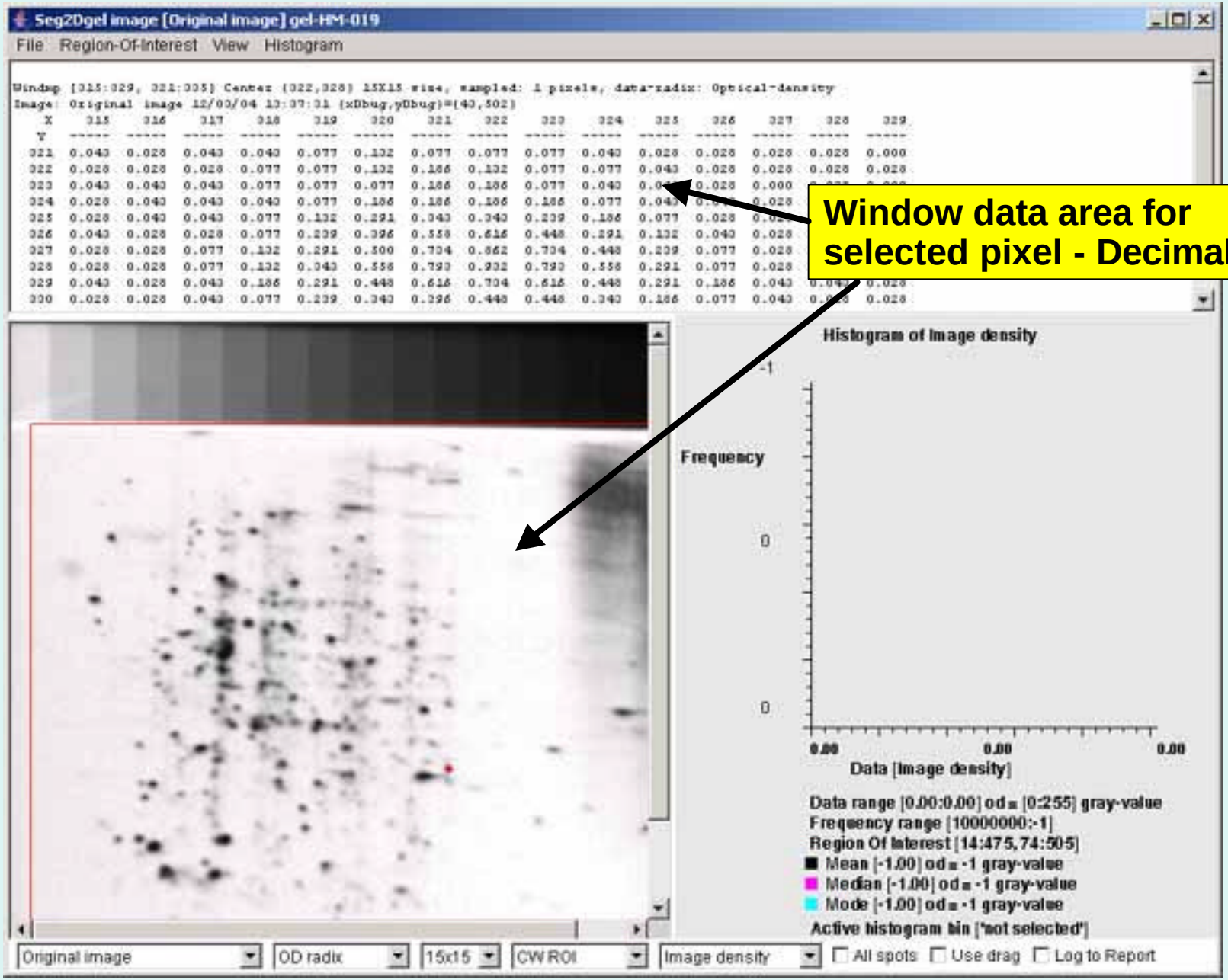
Seg2Dgel Report Window User Interface -gui mode



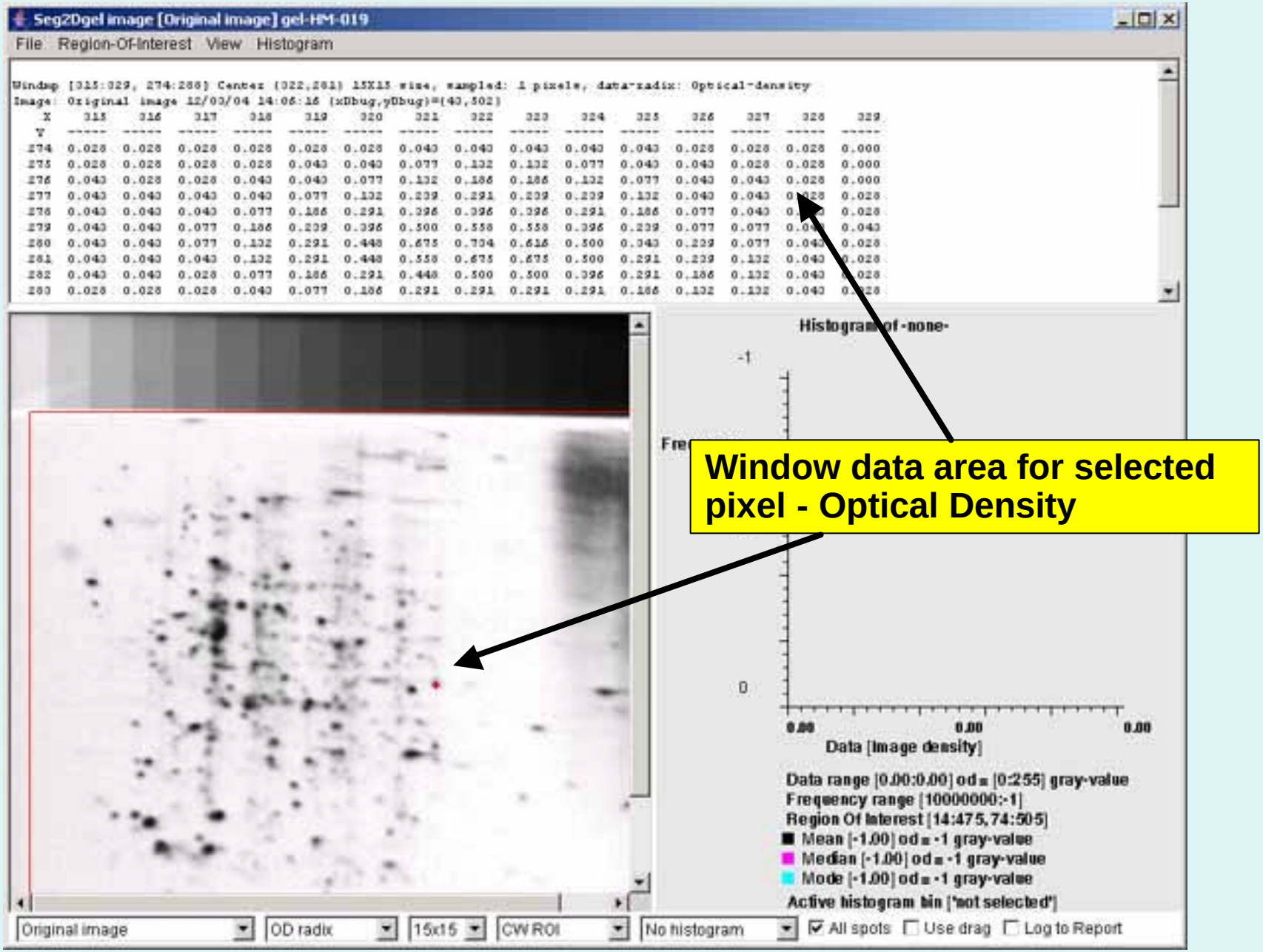
Seg2Dgel Command Options Wizard Window



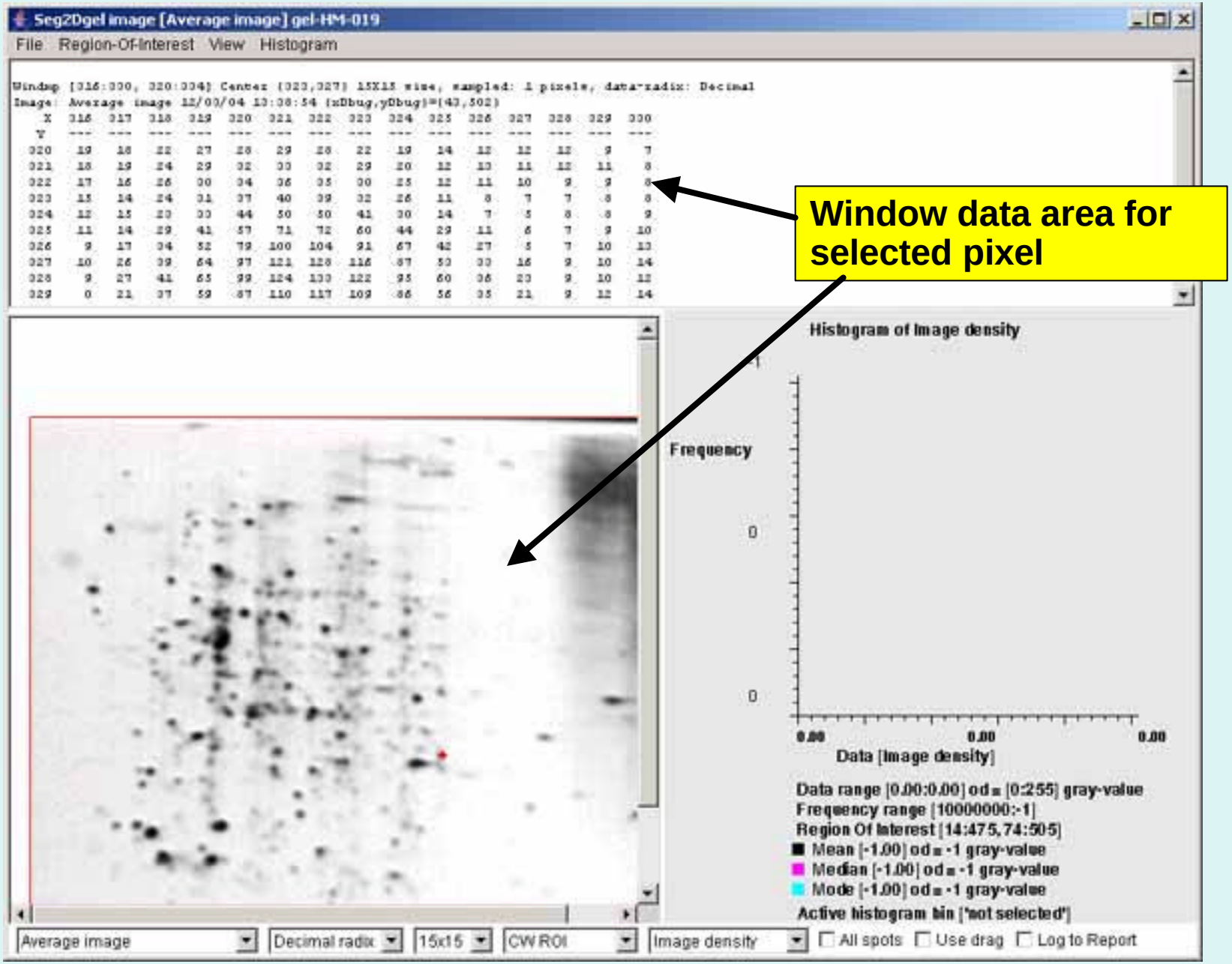
Seg2Dgel Image Viewer Window - original image - Decimal



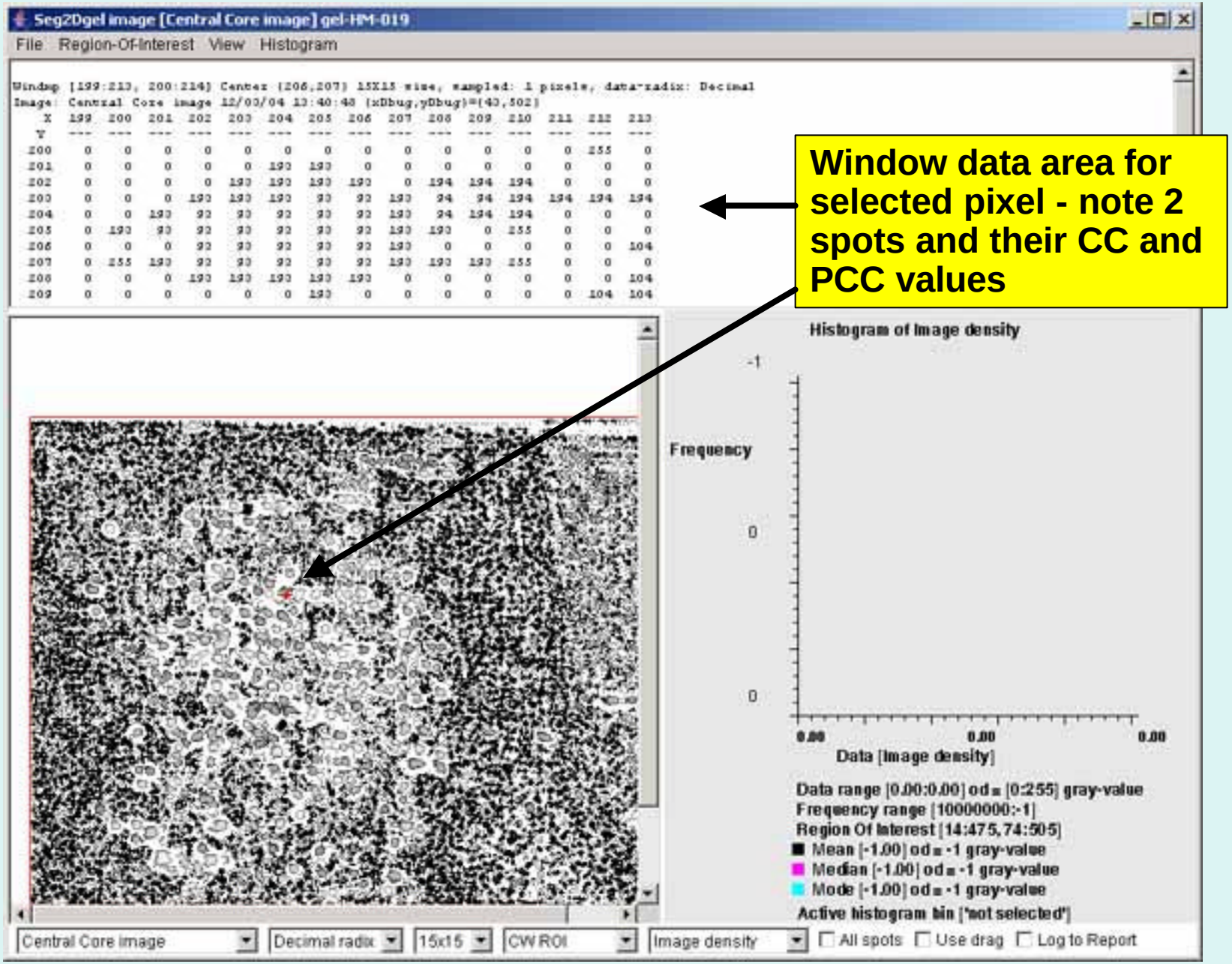
Seg2Dgel Image Viewer - original image - OD



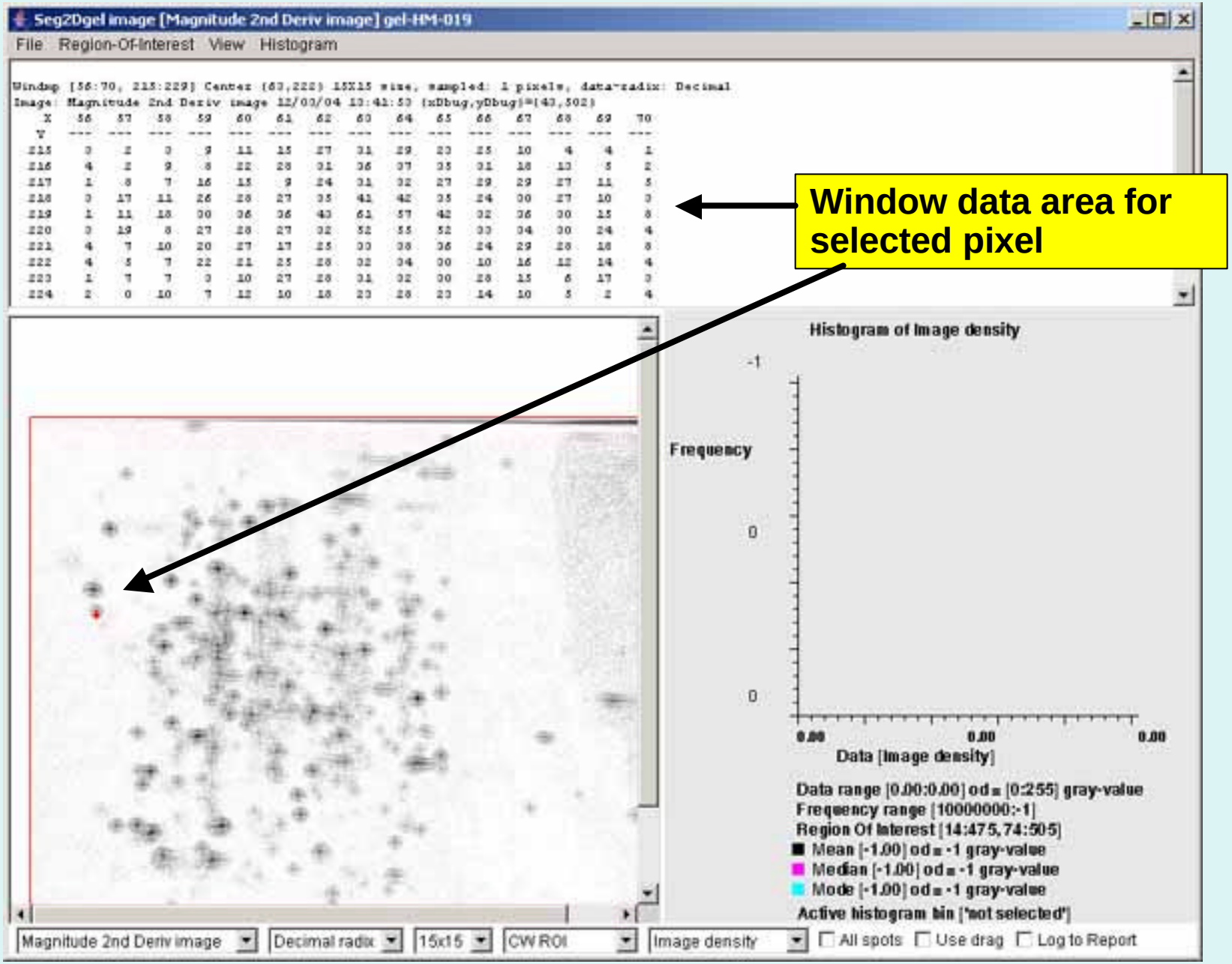
Seg2Dgel Image Viewer - Gaussian averaged image



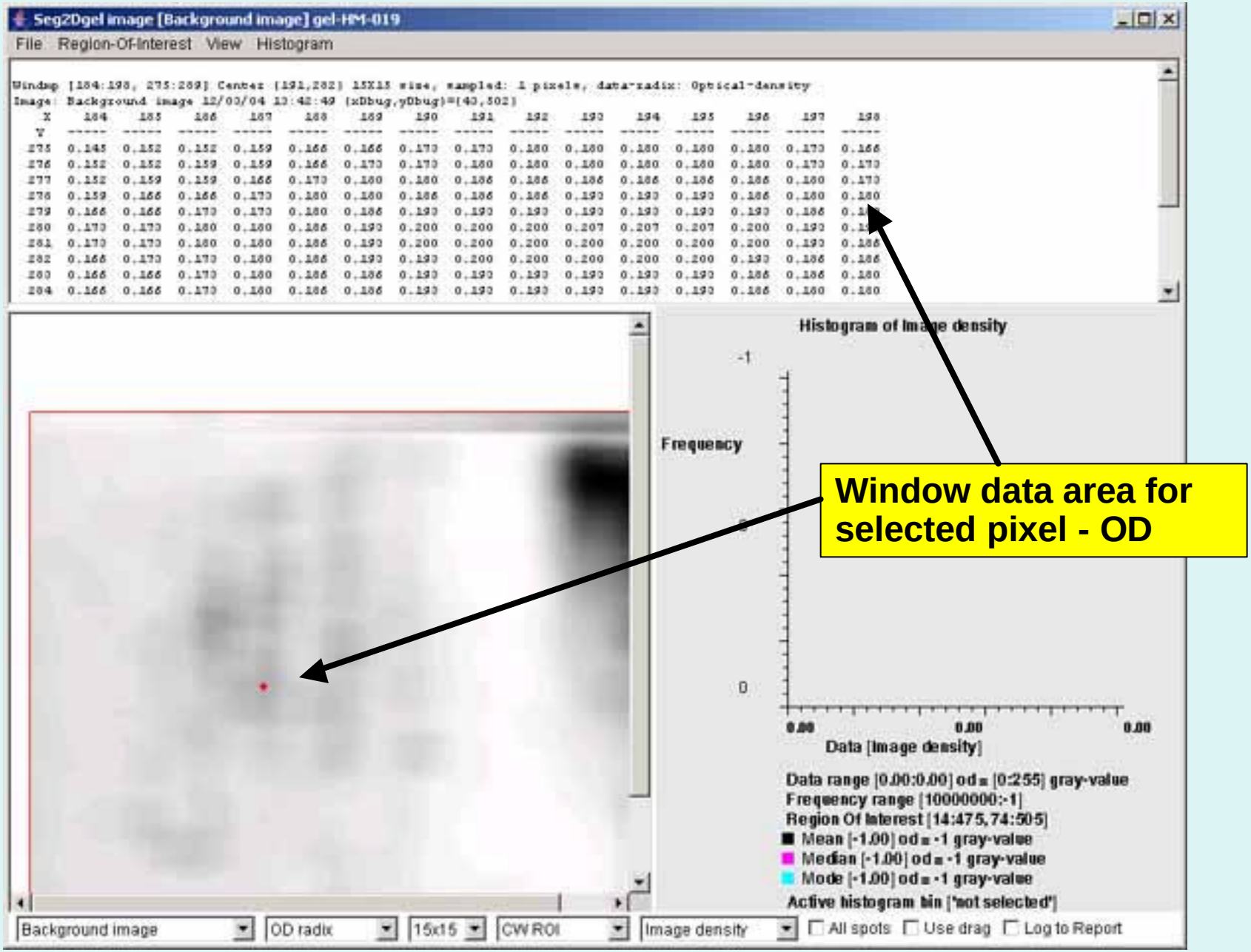
Seg2Dgel Image Viewer - Propagated Cental Core image



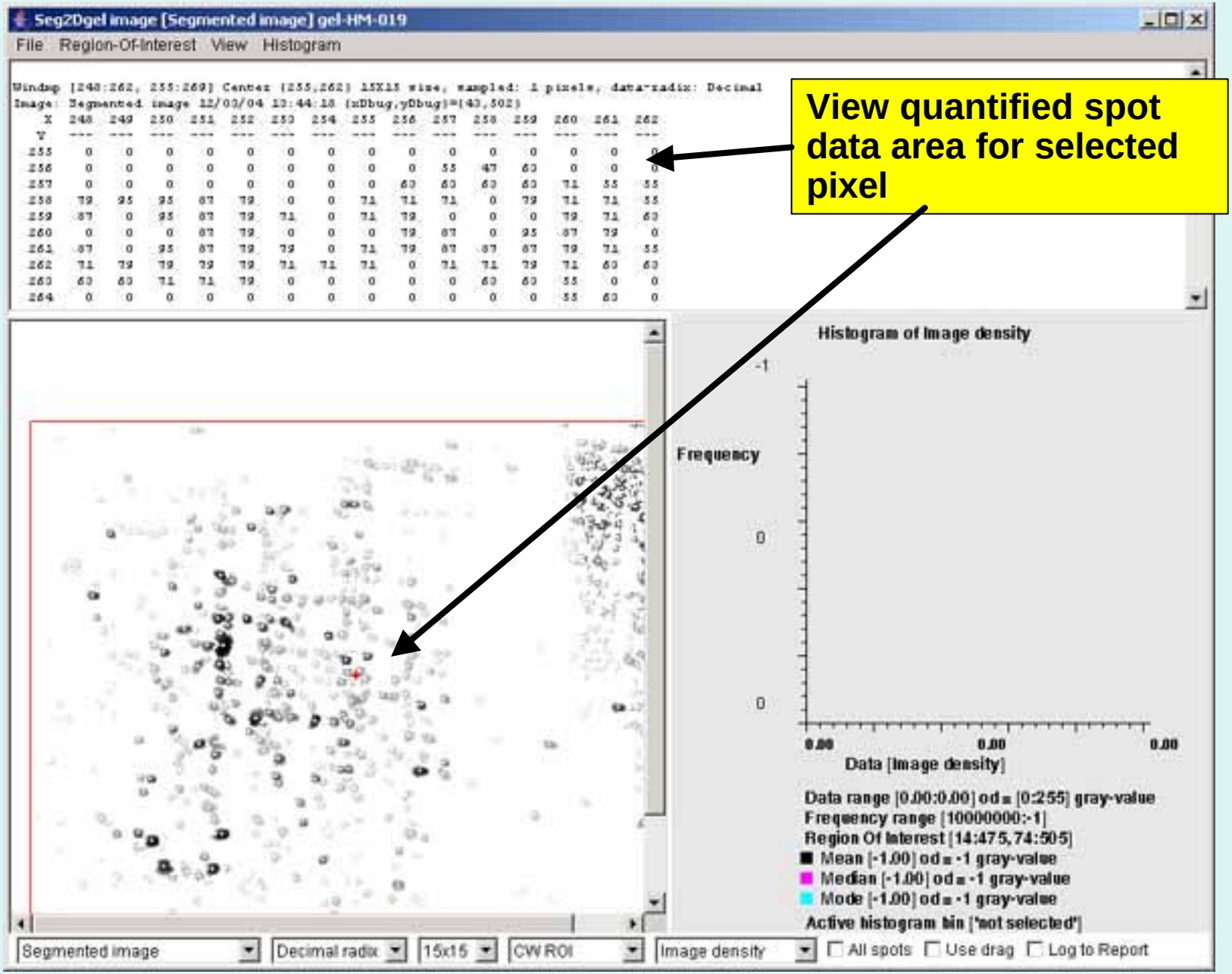
Seg2Dgel Image Viewer - Magnitude of 2nd Derivative image



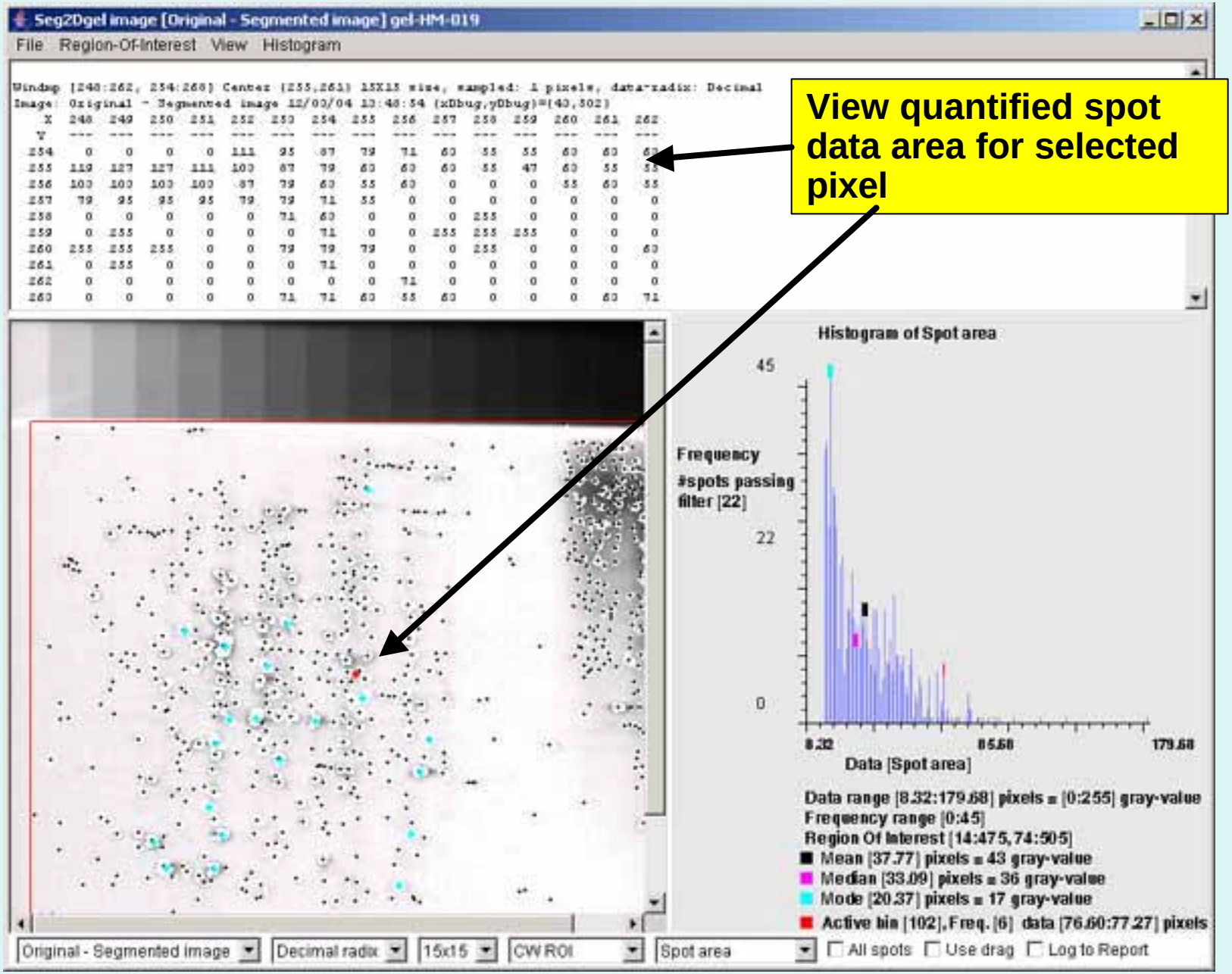
Seg2Dgel Image Viewer - background image - OD



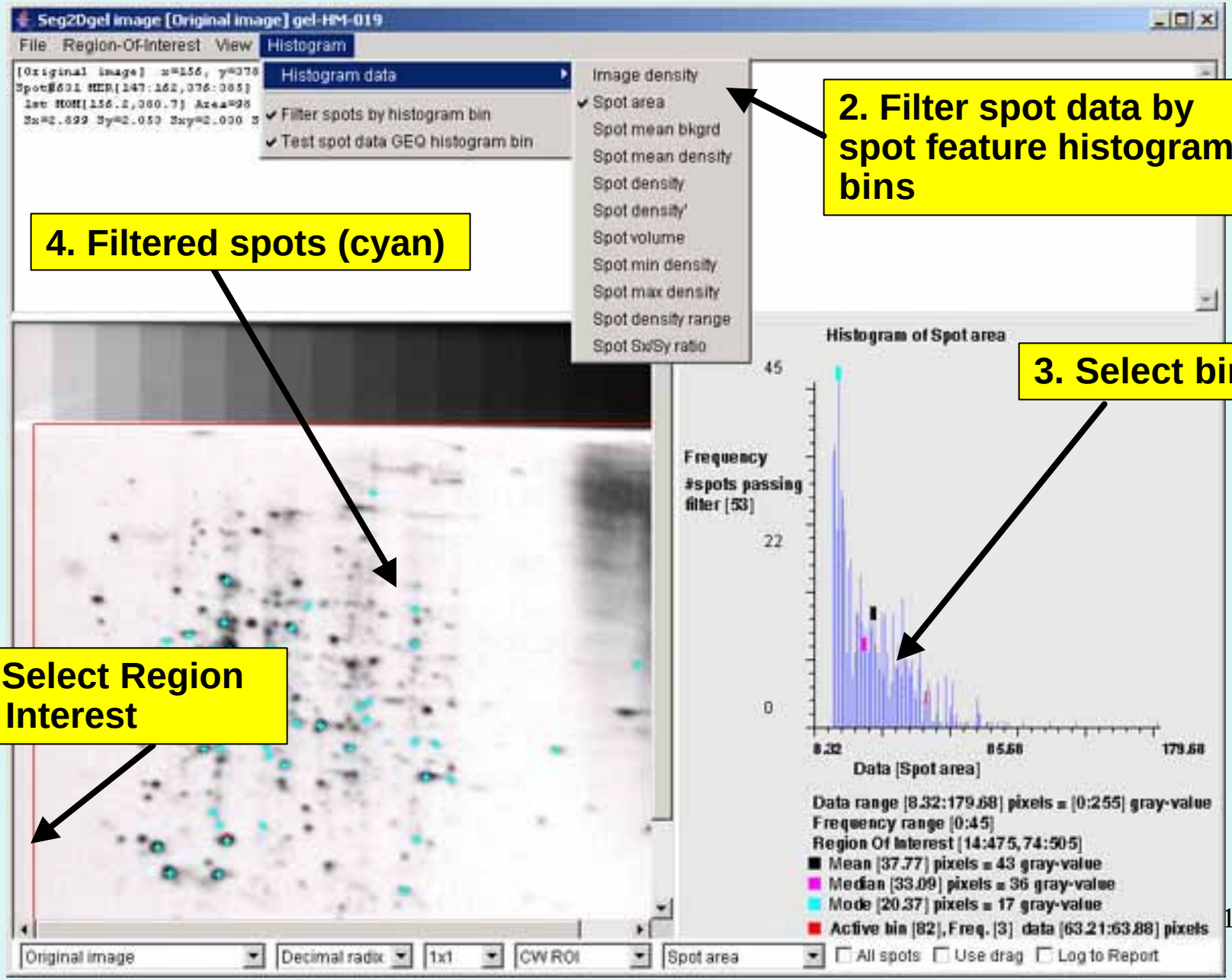
Seg2Dgel Image Viewer - segmented image



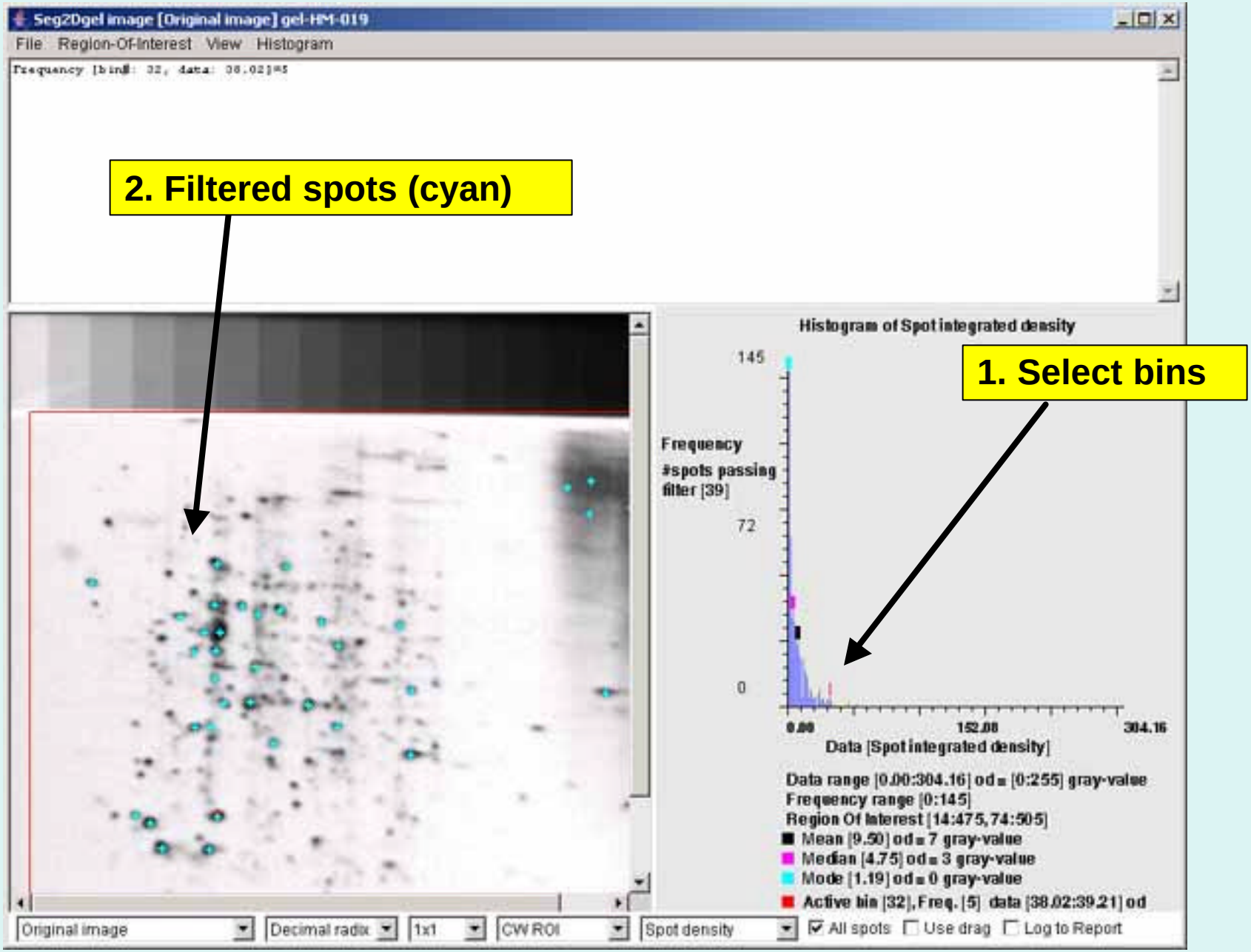
Seg2Dgel Image Viewer - rest of (original - segmented) image



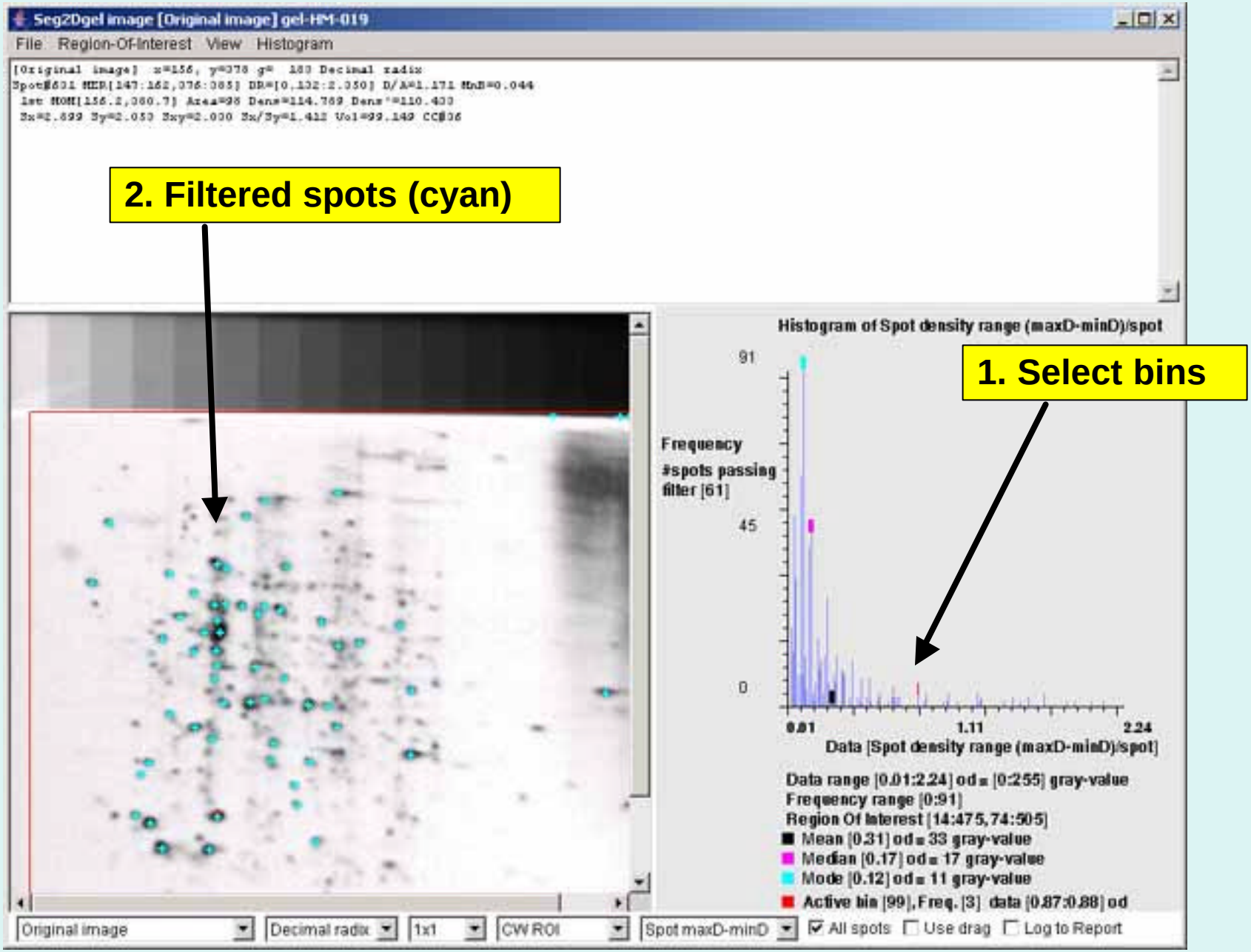
Seg2Dgel Image Viewer - with Histogram of spot area



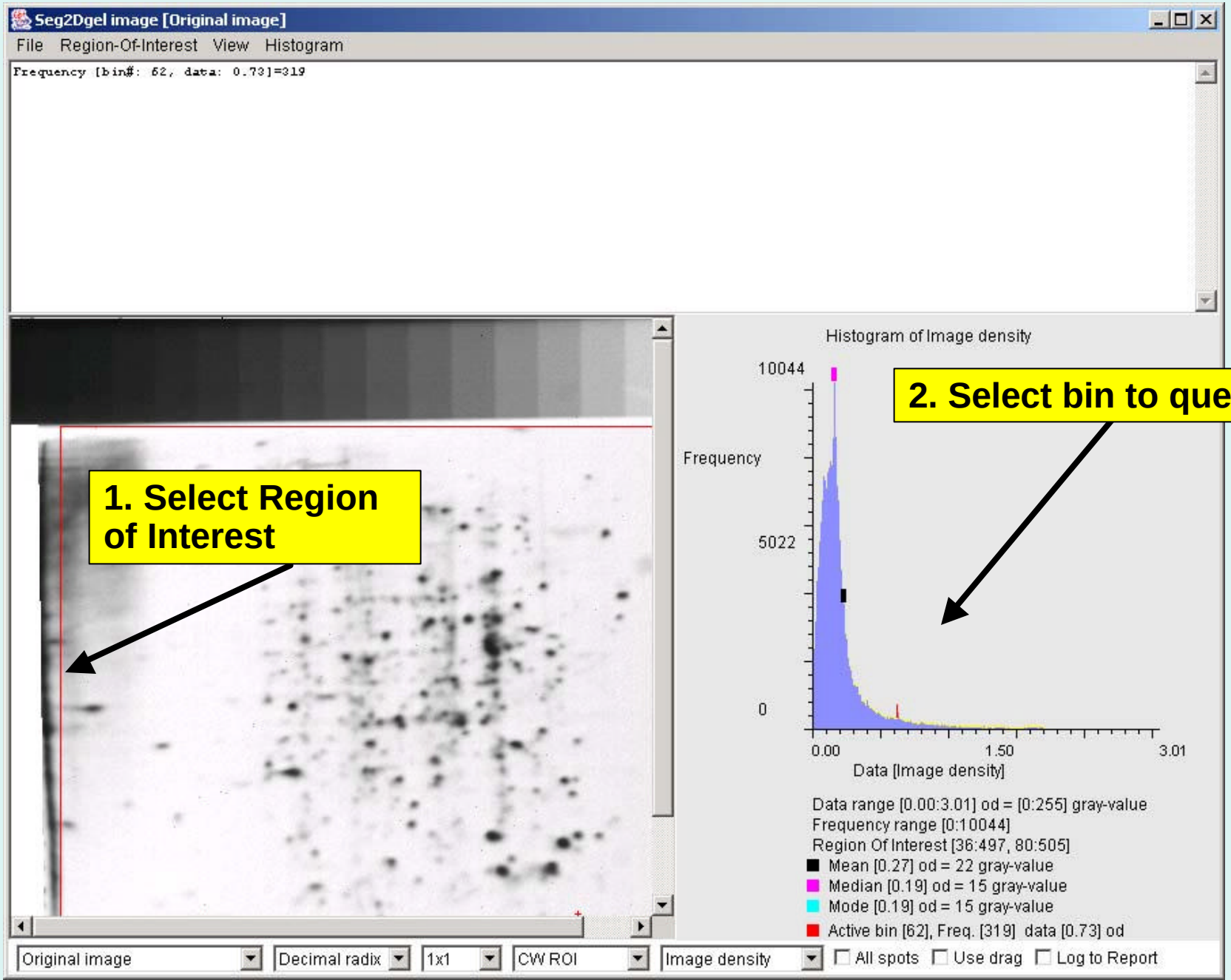
Seg2Dgel Image Viewer - Histogram of spot densities



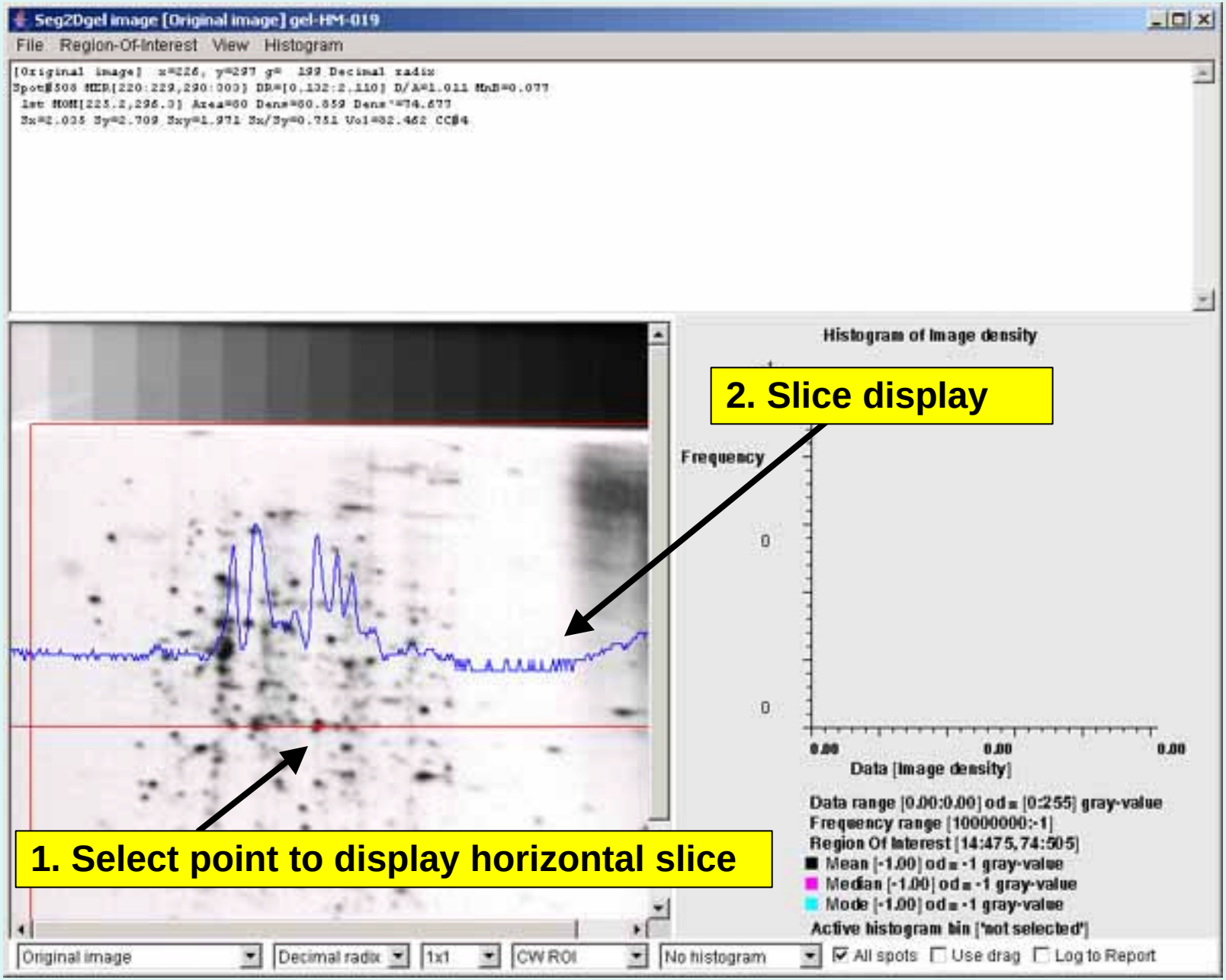
Seg2Dgel Image Viewer - Histogram of spot density-range



Seg2Dgel Image Viewer - Histogram of image densities



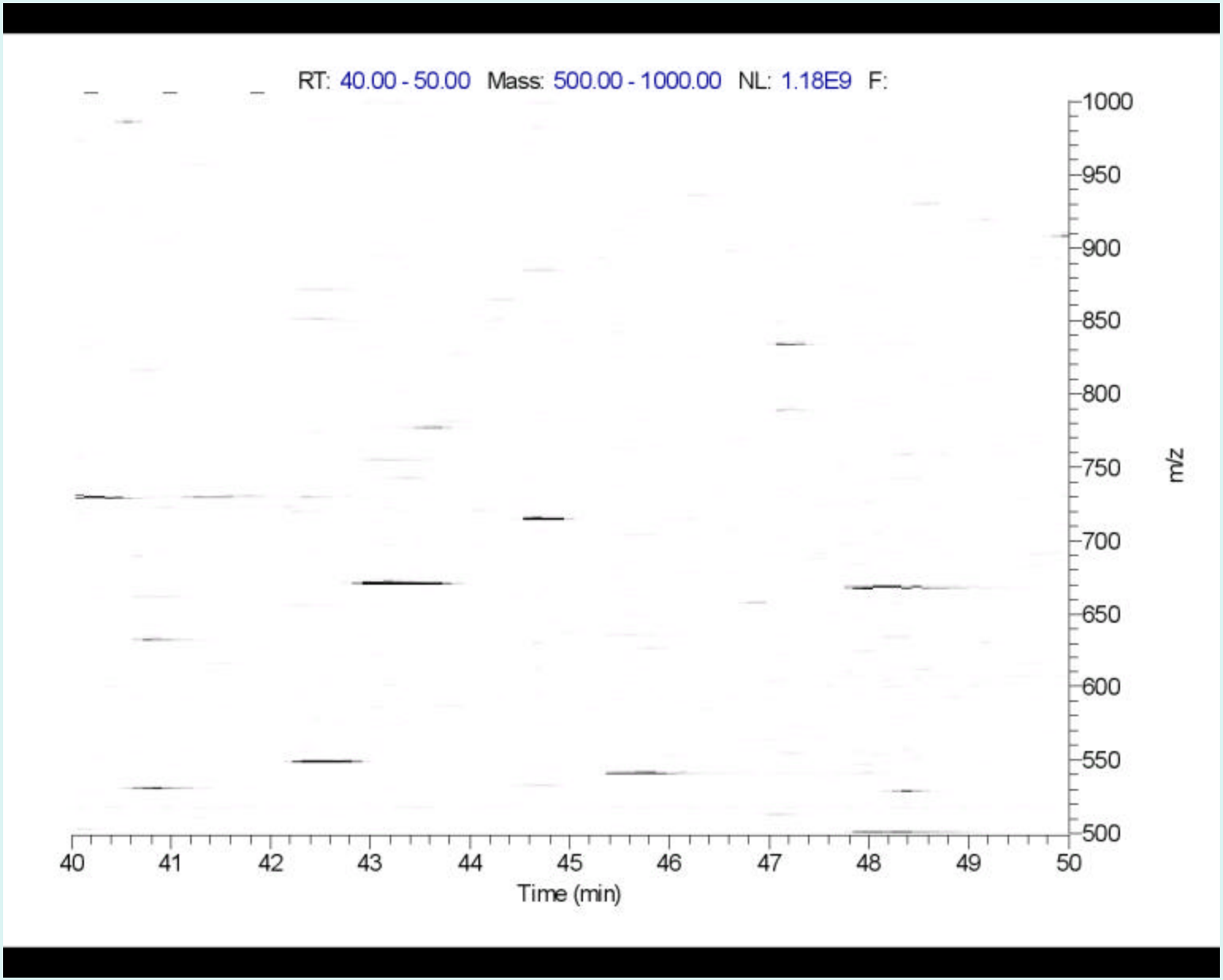
Seg2Dgel Image Viewer - Image density slice



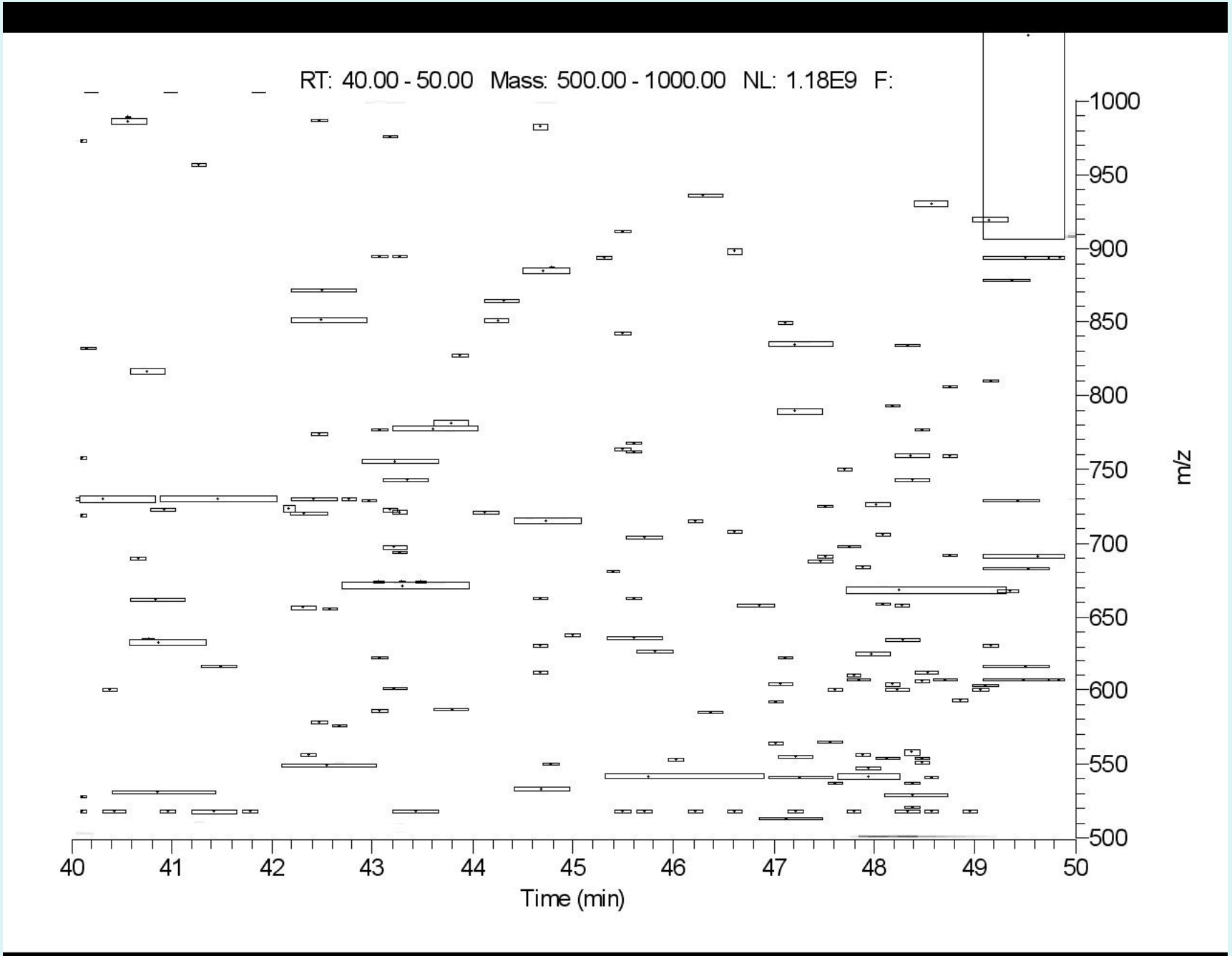
Using the Segmenter on 2D LC-MS Data

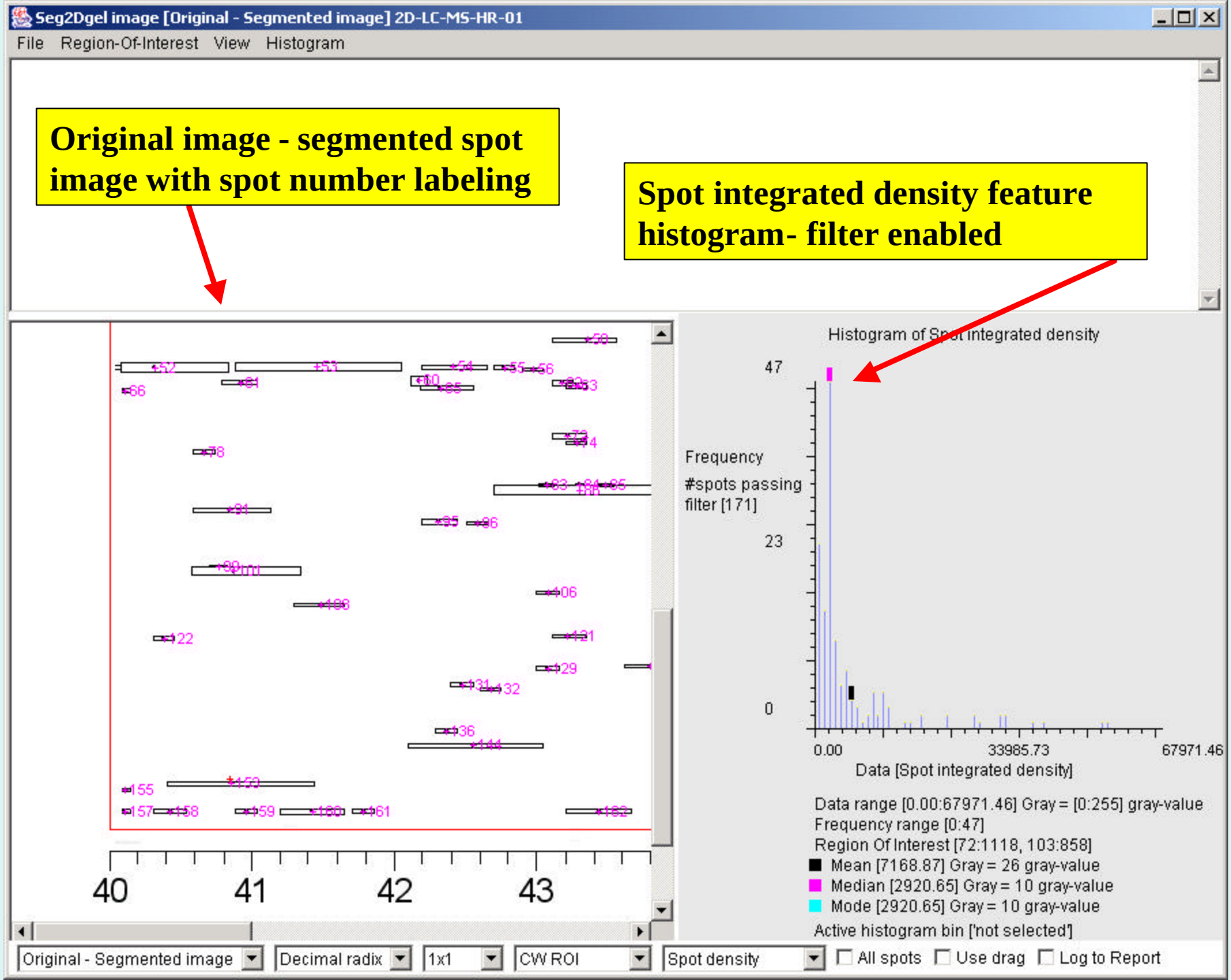
- The following slides show that the segmenter can be used with low resolution (~800x600 pixel image shown here) 2D LC-MS images
- Uses –laplacian:H,3,9 (horizontal Laplacian line filter)
- Prefilter with 3x3 Gaussian filter (-lowPass:3)
- View spot rectangles using –drawMinEnclosingRect
- Use -thrSxSxRatio: 2.5,100 to filter out small noisy line segments. This uses a density weighted spot X and Y deviation ratio Sx/Sy >= 2.5

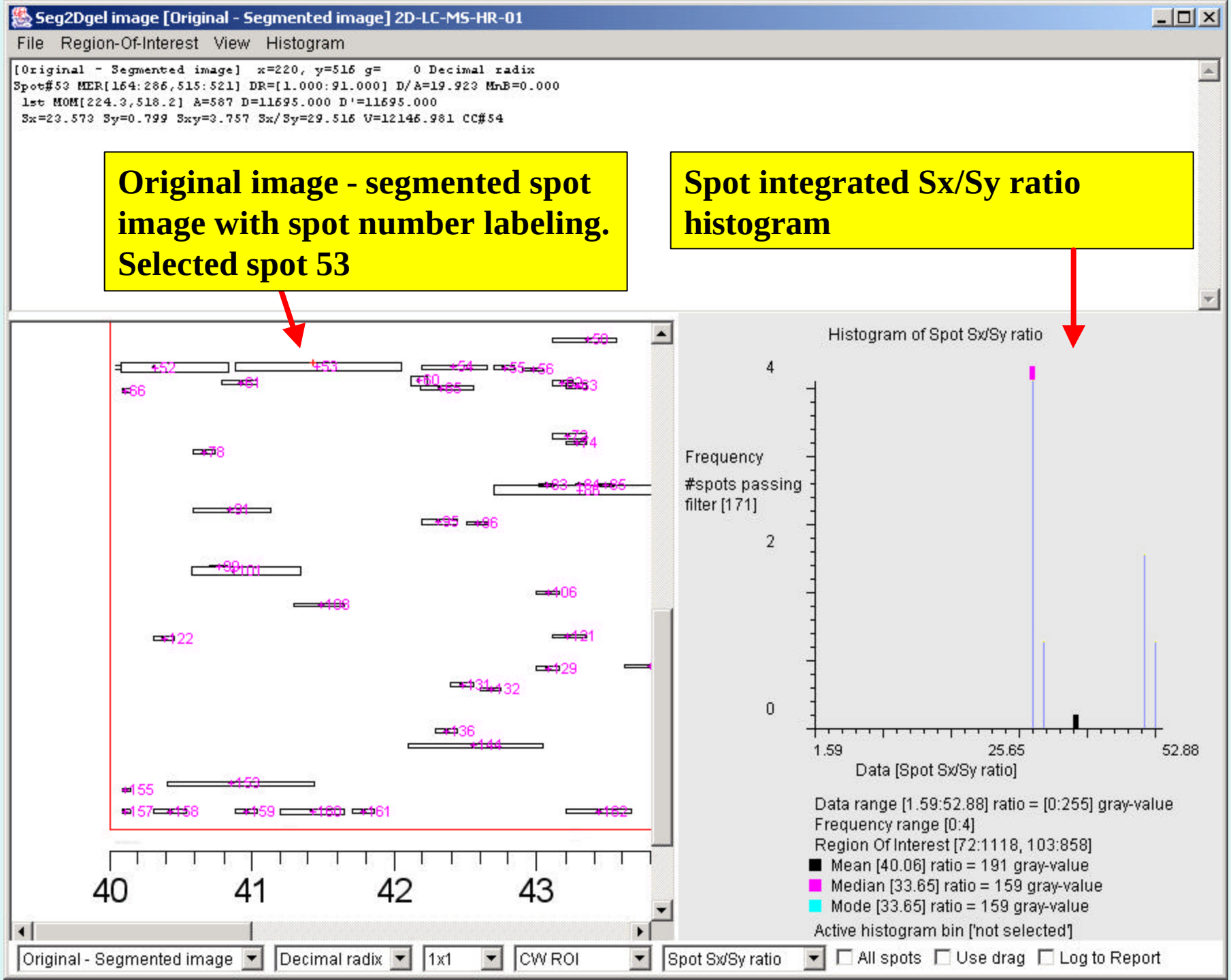
Original image: 2D-LC-MS-HR-01.gif



Original image - segmented image : 2D-LC-MS-HR-01-rest.gif




Seg2Dgel image [Original - Segmented image] 2D-LC-MS-HR-01
File Region-Of-Interest View Histogram
Original image - segmented spot image with spot number labeling
Spot integrated density feature histogram- filter enabled
Histogram of Spot integrated density
47
Frequency
#spots passing filter [171]
23
0
0.00
33985.73
67971.46
Data [Spot integrated density]
Data range [0.00:67971.46] Gray = [0:255] gray-value
Frequency range [0:47]
Region Of Interest [72:1118, 103:858]
Mean [7168.87] Gray = 26 gray-value
Median [2920.65] Gray = 10 gray-value
Mode [2920.65] Gray = 10 gray-value
Active histogram bin ['not selected']
40
41
42
43
Original - Segmented image
Decimal radix
1x1
CW ROI
Spot density
All spots
Use drag
Log to Report


Seg2Dgel image [Original - Segmented image] 2D-LC-MS-HR-01
File Region-Of-Interest View Histogram
[Original - Segmented image] x=220, y=516 g= 0 Decimal radix
Spot#53 MER[164:286,515:521] DR=[1.000:91.000] D/A=19.923 MnB=0.000
1st MOM[224.3,518.2] A=587 D=11695.000 D'=11695.000
Sx=23.573 Sy=0.799 Sxy=3.757 Sx/Sy=29.516 V=12146.981 CC#54
Original image - segmented spot image with spot number labeling. Selected spot 53
Spot integrated Sx/Sy ratio histogram
40
41
42
43
Histogram of Spot Sx/Sy ratio
Frequency
#spots passing filter [171]
4
2
0
1.59
25.65
52.88
Data [Spot Sx/Sy ratio]
Data range [1.59:52.88] ratio = [0:255] gray-value
Frequency range [0:4]
Region Of Interest [72:1118, 103:858]
Mean [40.06] ratio = 191 gray-value
Median [33.65] ratio = 159 gray-value
Mode [33.65] ratio = 159 gray-value
Active histogram bin ['not selected']
Original - Segmented image
Decimal radix
1x1
CW ROI
Spot Sx/Sy ratio
All spots
Use drag
Log to Report

Summary

- Seg2Dgel is an open-source 2D gel spot segmentation Java program freely available at **http://open2dprot.sourceforge.net/Seg2Dgel**

- Useful for segmenting spots in 2D gels and other images with similar types of data.

- It will be used as one of the step [2] alternative modules in the analysis pipeline in the Open2Dprot project at **http://open2dprot.sourceforge.net**